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SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM[1]

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DETERMINATION OF THE THEBAINE-CONTENT OF LEAVES OF PHYTOTRON-GROWN[2] PAPAVER BRACTEATUM[3] PLANTS

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1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ The cultivation of the P. bracteatum plants was carried out in the Laboratory of Plant Physiology, University of Geneva, under the supervision of Professor H. Greppin, in order to establish optimum conditions for in-door growing of P. bracteatum. For comparative purposes, out-door cultures were carried out under the supervision of Mr. G. Meylan and Mr. L. Wuarin.

3/ Origin of the seeds: P. bracteatum Arya I, Professor I. Lalezari, Department of Organic Chemistry, Faculty of Pharmacy, University of Teheran, Iran.

GE.74-9301

Since the publication of our report[4/] on the analysis of Papaver alkaloids and stability of thebaine, several attempts have been made to improve the extraction procedures and the GLC analysis of thebaine. Because of the instability of thebaine in acidic solutions,[4/] we considered it necessary to use basic solvent mixtures in order to avoid decomposition during extraction. An additional advantage of basic solvent mixtures, which extract the alkaloids in the form of tertiary amines, is the possibility of direct injection and analysis in the gas chromatograph.

In general, it is found that the chemical composition of leaves, roots and capsules is different and consequently the extraction procedures for the individual materials should also differ for optimal thebaine and/or total alkaloid extraction. For example, in the case of the leaves, a pre-purification step should be carried out in order to remove from the extract the chlorophylls and fats, which contaminate the injection ports of the GC system. Because of the lack of radioactive labelled thebaine - which would permit the measurement of the loss of thebaine during the pre-purification procedure - the crude chlorophyll-containing extract is presently still injected into the gas chromatograph as such and therefore the injection port has to be cleaned regularly. It is our present experience that particularly the 80-100 mesh size of the stationary phase support, chromosorb G coated with 3 per cent OV-17, provides excellent separation with complete absence of peak-tailing in routine analyses of thebaine-containing extracts. Using the above stationary phase, a low pre-pressure of 1.8 atm and a relatively low column temperature of 240°C, thebaine shows a retention time of about 15 minutes.

Comparison of the standard extraction procedure for leaves with an exhaustive Soxhlet-extraction

The following procedure was used for the routine extraction of air-dried leaf material of phytotron-grown P. bracteatum plants:

1. The material is ground in a mortar to pass through a 20 mesh-sieve.
2. 500 mg of the resulting powder is extracted for 16 hours at 25°C with 25 ml methanol.
3. The methanolic extract is decanted and evaporated to dryness using a rotatory evaporator.
4. The residue is extracted for 30 minutes at 50°C with 10 ml 2 per cent NH_4OH (spec. grav. 0.900) in methanol.
5. The extracts from 3 and 4 are combined, adjusted to a proper volume and analyzed by GLC.

A number of samples were divided in two equal parts. One part was extracted using the procedure described above and the other part was exhaustively extracted in a

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4/ F.J.E.M. Küppers, R.J.J.Ch. Lousberg, C.A.L. Bercht and C.A. Salemink United Nations document ST/SOA/SER.J/8.

Soxhlet extraction apparatus (extraction mixture: 2 per cent NH_4OH in methanol, duration: 24 hours, number of passages of extraction fluid 300). The results are given in Table I.

Table I

sample nr.	standard extraction % thebaine (A)	Soxhlet extraction % thebaine (B)	$\frac{A}{B}$ x 100%
1	0.165	0.175	94
2	0.505	0.540	93
3	0.340	0.360	94
4	0.550	0.585	94
5	0.225	0.240	93
6	0.085	0.090	94
7	0.280	0.295	95
8	0.105	0.110	95

These results indicate that the "routine extraction" is almost as effective as an exhaustive extraction and, because of the much smaller demand in time, energy and instrumental equipment, this procedure was applied to the phytotron-grown series of P. bracteatum plants. The results in the following tables are not corrected for the above mentioned difference of about 6 per cent.

Comparison of thebaine content determined in leaf material dried according to four different methods

Until now, no uniform method of drying fresh P. bracteatum samples has been used. In order to establish the most suitable way of drying, which would keep the decomposition of thebaine minimal, the following experiments were carried out:

Eleven phytotron-grown P. bracteatum samples, each containing at least five different plants, were divided into five equal parts. The four drying procedures applied to these samples were:

1. Freeze-drying / 24 hours
2. Oven-drying at 105° / 1 hour
3. Oven-drying at 60° / 3 hours
4. Air-drying at 25° / 48 hours

The fifth part of each series was placed in methanol without previous drying. After drying, all plant samples were extracted by the same "routine procedure" and analyzed by GLC. The results are given in table II.

Table II

sample	freeze-drying % theb.	oven 105°/1hr % theb.	oven 60°/3hr % theb.	air 5/ 25°/48hr % theb.	fresh in methanol 6/
1	0.28	0.25	0.14	0.05	0.01
2	0.17	0.15	0.10	0.06	"
3	0.40	0.33	0.23	0.13	"
4	0.24	0.21	0.18	0.14	"
5	0.30	0.26	0.22	0.20	"
6	0.56	0.48	0.41	0.31	"
7	0.22	0.13	0.11	0.11	"
8	0.39	0.32	0.30	0.28	"
9	0.55	0.48	0.44	0.37	"
10	0.12	0.10	0.09	0.08	"
11	0.58	0.55	0.52	0.46	"
mean	0.35	0.29	0.25	0.22	-

From the data in this table, it was concluded that freeze-drying is the most suitable method to be applied for quantitative thebaine analyses of leaf material.

Until now, the phytotron-grown P. bracteatum plants were sent from Geneva to Utrecht by plane without previous drying. It took about 48 hours between harvest and arrival at our Institute. Biological processes, which had been able to decrease the thebaine content, had thus already taken place. Rigorous freeze-drying could not further account for lower percentages of thebaine as found in table II.

Conditions for phytotron cultures of P. bracteatum

In the phytotron, the following schedule was applied:

a. variation of photo-period: 24, 20, 16 and 12 hours/day

b. variation of light intensity: 8000, 6000, 4000 and 2000 lux

During the photo-period the temperature was 20°C and the relative humidity 80 per cent. During the dark period, the temperature was also 20°C and the relative humidity 60 per cent. All plants were cultivated on artificial soil (terralit), nutrition 0.5 per cent Lonzin plus 0.5 per cent Wuxal. The results of these series of experiments are shown in Tables III and IV and in figures 1 and 2.

5/ Additional freeze-drying of this air dried material caused a further loss of 10 per cent in weight.

6/ The extraction time (2 weeks) proved to be too long; virtually all the thebaine decomposed.

The results indicate that, under the phytotron conditions applied, the combination of a photo-period of 24 hours and a light intensity of 6000 lux induces a maximum thebaine production in plants of about 160 days old, whereas a photo-period of 20 hours/6000 lux gives a maximum thebaine content in plants 130 days old. Both 16 and 12 hours of photo-period seem less favourable for thebaine production.
Note: The values, obtained with 102 day old plants (Table III) all originate from one series of harvest. This series was analyzed on a OV-1 column under apparently less favourable conditions and may have yielded too low results.

In addition to thebaine, substantial amounts of at least two other products were observed in every sample. In the gas chromatogram, their relative retention times were 0.77 and 1.44. Further structural analyses of these compounds will be the subject of a forthcoming report.

Table III

	age of plant at harvest (days)	% thebaine	
		photo-period 24 hr/day	photo-period 20 hr/day
photo-intensity 2000 lux	111	0.16	0.06
	131	0.30	0.30
	151	0.22	0.10
	182	0.16	0.17
	202	0.14	0.26
	237	0.17	0.32
photo-intensity 4000 lux	111	0.12	0.17
	131	0.34	0.36
	151	0.26	0.10
	182	0.24	0.14
	202	0.31	0.20
	237	0.27	0.35
photo-intensity 6000 lux	111	0.15	0.32
	131	0.50	0.55
	162	0.55	
	169		0.05
	182	0.49	0.15
	202	0.31	0.33
	209	0.29	
	237		0.38
photo-intensity 8000 lux	111	0.25	0.16
	131	0.49	0.40
	162	0.45	0.29
	182	0.23	0.22
	202	0.21	0.27
	237		0.30

Table IV

	age of plant at harvest (days)	% thebaine	
		photo-period 16 hr/day	photo-period 12 hr/day
photo-intensity 4000 lux	145	0.20	0.14
	169	0.23	0.13
	196	0.09	0.21
	216	0.20	0.19
	223	0.21	0.09
photo-intensity 6000 lux	145	0.27	0.16
	169	0.09	0.12
	196	0.18	0.24
	216	0.28	0.20
	223	0.15	
photo-intensity 8000 lux	145	0.16	0.13
	169	0.11	0.16
	196	0.08	0.17
	216	0.29	0.16
	223	0.22	0.11

Acknowledgments

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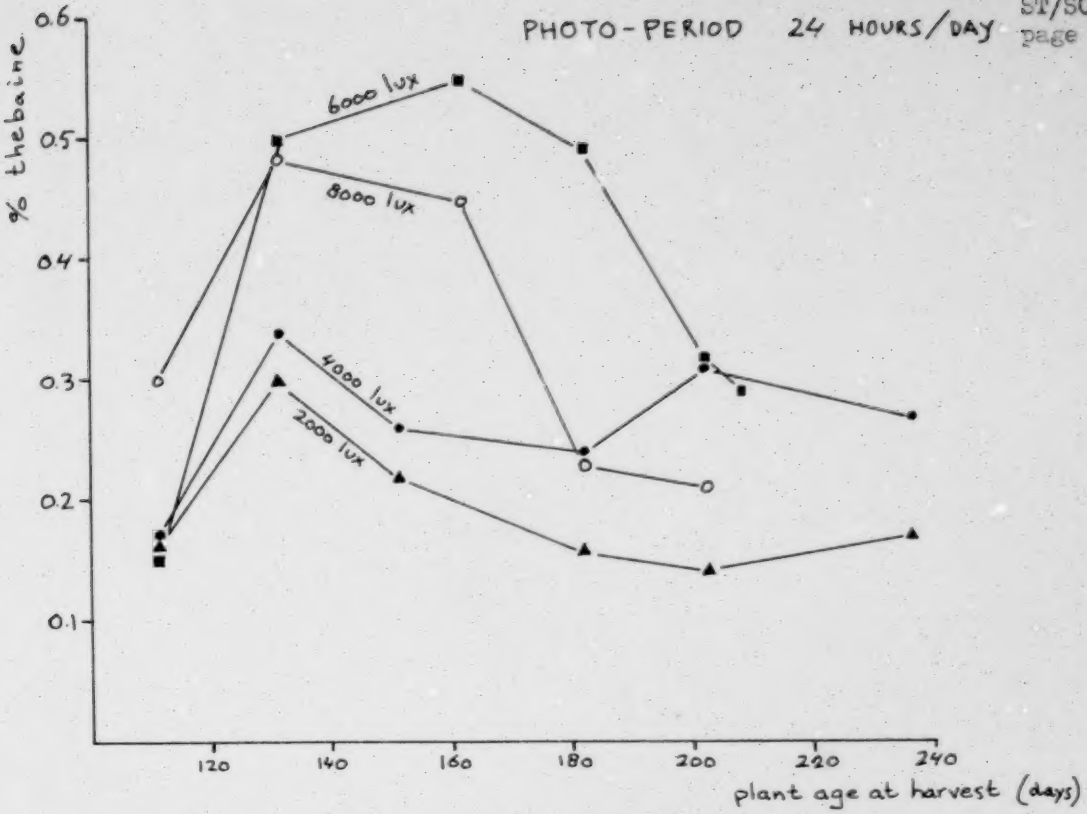
PHOTO-PERIOD 24 HOURS/DAY
% thebaine
0.6
0.5
0.4
0.3
0.2
0.1
6000 lux
8000 lux
4000 lux
2000 lux
120
140
160
180
200
220
240
plant age at harvest (days)

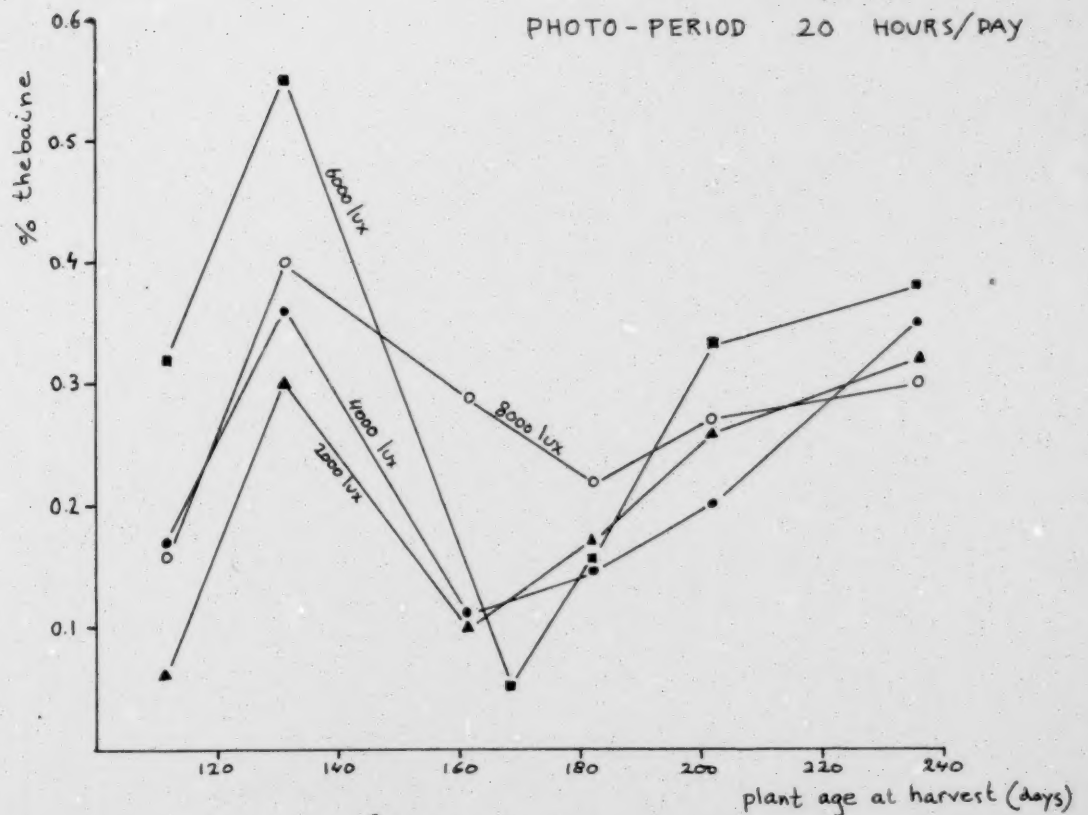
PHOTO-PERIOD 20 HOURS/DAY
% thebaine
0.6
0.5
0.4
0.3
0.2
0.1
6000 lux
8000 lux
4000 lux
2000 lux
120
140
160
180
200
220
240
plant age at harvest (days)

figure 1

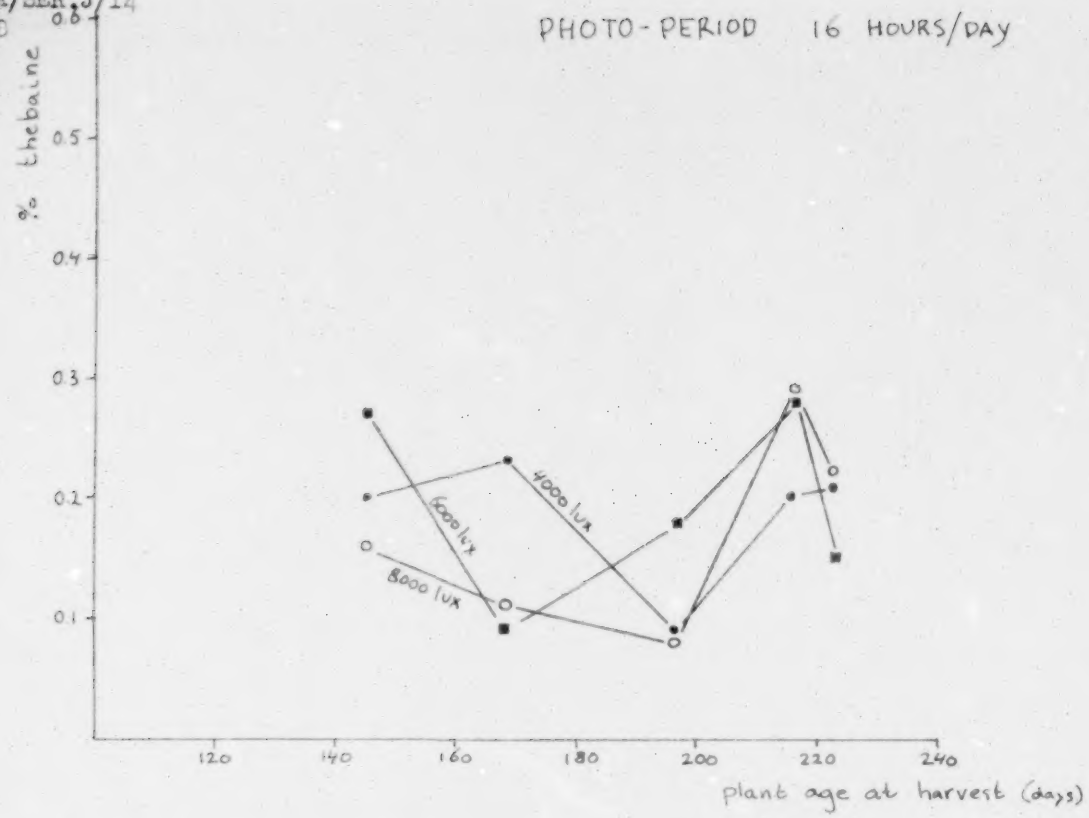
PHOTO-PERIOD 16 HOURS/DAY
% thebaine
0.6
0.5
0.4
0.3
0.2
0.1
6000 lux
4000 lux
8000 lux
120
140
160
180
200
220
240
plant age at harvest (days)

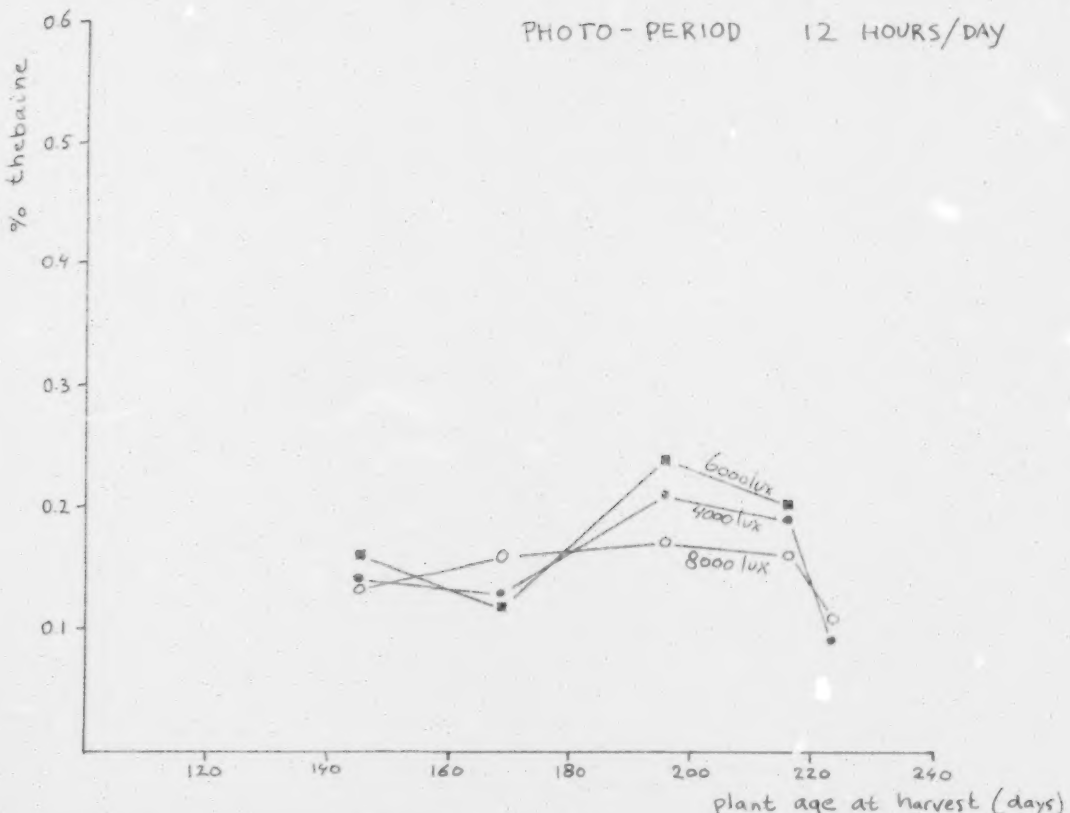
PHOTO-PERIOD 12 HOURS/DAY
% thebaine
0.6
0.5
0.4
0.3
0.2
0.1
6000 lux
4000 lux
8000 lux
120
140
160
180
200
220
240
plant age at harvest (days)

figure 2